
Dissecting direct reprogramming from fibroblast to neuron using single-cell RNA-seq.

Journal: Nature

Publication Year: 2016

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PubMed link: 27281220

Funding Grants: Mechanisms of human induced neuronal cell reprogramming

Public Summary:

Direct lineage reprogramming represents a remarkable conversion of cellular and transcriptome states. However, the intermediate stages through which individual cells progress during reprogramming are largely undefined. Here we use single-cell RNA sequencing at multiple time points to dissect direct reprogramming from mouse embryonic fibroblasts to induced neuronal cells. By deconstructing heterogeneity at each time point and ordering cells by transcriptome similarity, we find that the molecular reprogramming path is remarkably continuous. Overexpression of the proneural pioneer factor *Ascl1* results in a well-defined initialization, causing cells to exit the cell cycle and re-focus gene expression through distinct neural transcription factors. The initial transcriptional response is relatively homogeneous among fibroblasts, suggesting that the early steps are not limiting for productive reprogramming. Instead, the later emergence of a competing myogenic program and variable transgene dynamics over time appear to be the major efficiency limits of direct reprogramming. Moreover, a transcriptional state, distinct from donor and target cell programs, is transiently induced in cells undergoing productive reprogramming. Our data provide a high-resolution approach for understanding transcriptome states during lineage differentiation.

Scientific Abstract:

Direct lineage reprogramming represents a remarkable conversion of cellular and transcriptome states. However, the intermediate stages through which individual cells progress during reprogramming are largely undefined. Here we use single-cell RNA sequencing at multiple time points to dissect direct reprogramming from mouse embryonic fibroblasts to induced neuronal cells. By deconstructing heterogeneity at each time point and ordering cells by transcriptome similarity, we find that the molecular reprogramming path is remarkably continuous. Overexpression of the proneural pioneer factor *Ascl1* results in a well-defined initialization, causing cells to exit the cell cycle and re-focus gene expression through distinct neural transcription factors. The initial transcriptional response is relatively homogeneous among fibroblasts, suggesting that the early steps are not limiting for productive reprogramming. Instead, the later emergence of a competing myogenic program and variable transgene dynamics over time appear to be the major efficiency limits of direct reprogramming. Moreover, a transcriptional state, distinct from donor and target cell programs, is transiently induced in cells undergoing productive reprogramming. Our data provide a high-resolution approach for understanding transcriptome states during lineage differentiation.

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